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MINI-REVIEW ARTICLE

State-of-the-art Application of Artificial Intelligence to Transporter-centered Functional and Pharmaceutical Research

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Abstract: Protein transporters not only have essential functions in regulating the transport of endogenous substrates and remote communication between organs and organisms, but they also play a vital role in drug absorption, distribution, and excretion and are recognized as major determinants of drug safety and efficacy. Understanding transporter function is important for drug development and clarifying disease mechanisms. However, the experimental-based functional research on transporters has been challenged and hinged by the expensive cost of time and resources. With the increasing volume of relevant omics datasets and the rapid evolution of artificial intelligence (AI) techniques, next-generation AI is becoming increasingly prevalent in the functional and pharmaceutical research of transporters. Thus, a comprehensive discussion on the state-of-the-art application of AI in three cutting-edge directions was provided in this review, which included (***a***) transporter classification and function annotation, (***b***) structure discovery of membrane transporters, and (***c***) drug-transporter interaction prediction. This study provides a panoramic view of AI algorithms and tools applied to the field of transporters. It is expected to guide a better understanding and utilization of AI techniques for in-depth studies of transporter-centered functional and pharmaceutical research.

Keywords: Transporter, artificial intelligence, machine learning, deep learning, functional annotation, structure, drug-transporter interaction.

1. INTRODUCTION

Protein transporters are recognized as 'gatekeepers' on the cell membrane and not only have important endogenous functions in regulating the transport of rate-limiting substrates (such as metabolites, signaling molecules, antioxidants, neurotransmitters, and bile salts) and remote communication between the organ and the organism [1-3], but also play a crucial function in the drug absorption, distribution, metabolism, and excretion (ADME), and are recognized as key determinants of drug safety and efficacy [4-8]. The transporters are inextricably interconnected with the drug efficacy and safety, as well as disease mechanisms, specifically, (***a***) the specific functional annotation of transporters is a critical aspect of substrates disposition *in vivo* and revealing the underlying molecular mechanisms of diseases [9-11]; (***b***) the structural information on transporters is vital to revealing drug resistance mechanisms and designing new drug entities [12-14]; (***c***) the interaction between drugs and transporters is key in identifying potential therapeutic targets or rational drug use [15-17]. Due to the unique role of transporters in cell communication and drug discovery, functional research on transporters is now proliferating.

However, the current studies on the above research fields of transporters are still facing enormous challenges. Particularly, with the advent of high-throughput omics data, a wealth of extensive information on candidate transporter proteins has been generated, but due to the high cost of time and resources of laboratory biological experiments, the growing need for functional annotation of transporters from large omics data could not be satisfied by experimental means alone [18]. Moreover, transporters were usually complex integral membrane proteins that were thus difficult to express, purify and detect for corresponding structural biological research, making it difficult to elucidate the molecular interactions between small molecules and transporter proteins, which hindered the development of effective drugs targeting transporters [19]. Furthermore, the lack of in-depth mining of the extensive transporter-related data has limited the systematic understanding of the biological processes of transporter and hindered the investigation of transporter-induced drug-drug interaction (DDI) and corresponding disease mechanism [4]. All in all, transporters are still clinically important proteins that have not been well studied due to the aforementioned challenges. In order to unravel the mechanisms underlying the important role of transporters in drug pharmacokinetics and disease mechanism, more effective tools are needed to further investigate transporter structure, functional characteristics, and drug-transporter interactions [1, 10, 20].

With the rapidly advancing artificial intelligence (AI) technology and the generation of large-scale data sets, the next-generation AI, especially deep learning (DL) methods, are gaining prevalence in pharmaceutical fields [21-26]. For example, AlphaFold, the well-known protein structure prediction tool that incorporates both physical and biological information on the protein structure into the design of novel DL algorithms, has made revolutionary advances in protein structure prediction with ongoing implications for the current development of novel drugs [27, 28]. Moreover, novel protein-coding strategies and methods of converting protein sequences into digital input models and AI algorithms have been developed for protein function annotation and protein-ligand interaction prediction to facilitate rational drug design, drug side-effects prediction, and structure-based target prediction [29-32]. The next-generation

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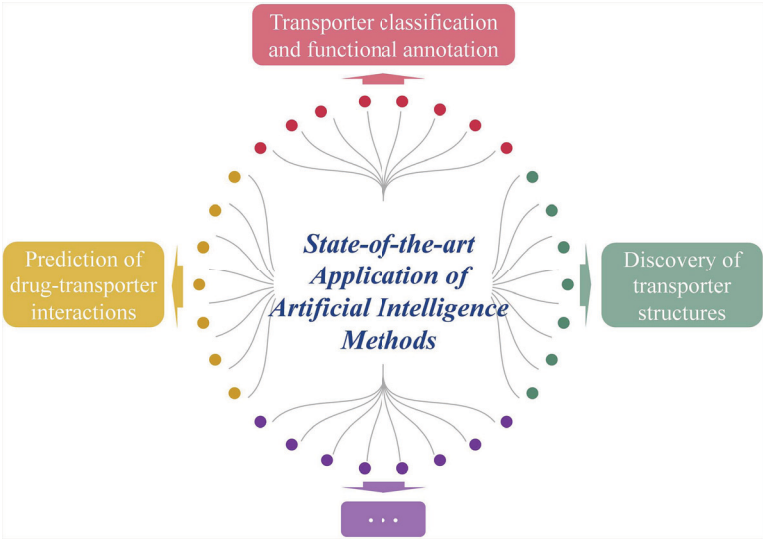

Fig. (1). An overview of the state-of-the-art application using artificial intelligence for transporter-centered functional and pharmaceutical research. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

AI approaches have rapidly emerged as the applicable solution to challenges in transporter functional annotation, transporter structure discovery, and drug transporter interaction prediction [33, 34].

Therefore, in this review, a comprehensive discussion on the state-of-the-art application of AI for (***a***) transporter classification and functional annotation, (***b***) transporter structure discovery, and (***c***) prediction of transporter-drug interactions were conducted, as shown in Fig. (**1**). Specifically, this study provides an introduction to the latest AI technologies and an overview of particular applications in these three areas, which is expected to provide supportive guidance for in-depth studies of transporters using AI technologies and offer practical insights for new drug development and drug target discovery.

2. APPLYING AI TECHNIQUE FOR ANNOTATING TRANSPORTER FUNCTION

The classification and functional annotation of transporters is essential to understanding and improving the *in vivo* ADME process of drugs, revealing disease mechanisms as well as drug resistance [18, 35]. Although the sequences of most general membrane proteins have been identified and deposited in public databases, such as TCDB [35] and TransportDB [36], there are still about 30% of the transporter family that is incompetently characterized or absent of experimental validation [37]. The significant imbalance between available transporter sequences and experimentally validated transporter functions is creating barriers to progress in drug discovery [38]. Advanced computational techniques were, therefore, adopted for the classification and the functional annotation of transporters to provide clues about further experimental protein function research.

Traditional predictions for the classification and function annotation of the transporter are mainly based on sequence similarity, such as BLAST and HMMER [30]. BLAST is a well-establish tool for discovering similar regions between protein sequences [30]. And HMMER is used to search the sequence databases for sequence homologs, and sequence alignment [39]. However, these sequence-similarity-based protein classifications and function predictions face the challenge of a high false discovery rate. The homology between the sequences of proteins could not guarantee their functional similarity. In other words, proteins with high sequence similarity did not always have a similar function [38, 40]. The rapidly evolving AI algorithms are expected to address the challenges of traditional sequence-similarity-based prediction of transporter classification and functional annotation [41]. Recently, various AI models, including convolutional neural networks (CNN), natural language processing (NLP), random forest (RF), support vector machine (SVM), *etc*., have been widely applied to the classification and functional annotation of transporters [42]. This section gives an overview of popular AI models that have achieved high performance in recent studies, and a detailed description of the AI algorithms, the feature extraction methods, and the datasets adopted for the specific method are presented in Table **1**. As well as the principles of various AI algorithms that have been widely used in the field of transporter function annotation and their specific application scenarios are shown in Fig. (**2**).

2.1. Support Vector Machine (SVM)

The SVM classifier is a machine learning algorithm that classifies labels in one or even higher dimensions [43] to create a boundary of decisions between two categories and predict features from one or more characteristic vectors [44]. The SVM has been widely applied as a classifier for cancer classification [45], drug discovery [46], and biomarker selection [47]. The following are the recent models using the SVM algorithm for transporter classification and function prediction.

ActTRANS [48] was constructed by integrating a bidirectional encoder representation based on contextual word embedding and SVM algorithm for classifying active transporters from transmembrane transport proteins. This tool allows the identification of specific amino acid residues in protein sequences and the extraction of feature vectors from hidden layers. Moreover, the tool has good performance for active transporter classification with an accuracy of 92.84%. The results indicate that the method can effectively classify active transporters and provides better performance than other feature extraction methods that use contextual information.

SCMMTP [49] is a computational tool for analyzing available protein sequences to identify membrane transporters. This tool presents a new method based on a propensity score using dipeptides, with membrane transporters being recognized and characterized from an available dataset of 900 membrane transporters and 660 non-membrane transporters, which were split into a training dataset of 1380 proteins and a test dataset of 180 proteins. The position-specific scoring matrix (PSSM) was the feature extraction method for membrane transporters in this method, yielding results for a test set accuracy of 80.56%.

Table 1. Artificial intelligence tools and models are applied to transporter classification and functional annotation. A detailed description of the AI algorithms, the feature extraction methods, and the datasets adopted for the specific methods are presented.

AI Methods	Model	Feature Extraction Methods (s)	Database(s) Employed	Year
Support Vector Machine (SVM)	ActTRANS [48]	Contextual relations between amino acids in the protein sequence	UniProtKB Database	2021
	SCMMTP [49]	Position-specific scoring matrix (PSSM)	Five datasets based on different sources of transporter proteins from various species	2015
	DeepNF [50]	Multimodal deep autoencoders	STRING database	2018
Random Forest (RF)	Hou's model [57]	188 features based on protein sequence and physical and chemical properties	UniProtKB database, Pfam database	2020
	Ebrahimie's model [40]	893 features using the PROFEAT, CLC Genomics Workbench, and ExPASy	UniProtKB Database	2021
	Ru's model [58]	The distance-based Top-n-gram method	UniProtKB database, Pfam database	2019
Convolutional Neural Network (CNN)	DeepIon [63]	Position-specific scoring matrix (PSSM)	UniProtKB database	2019
	Zhang's model [64]	CNN-based amino acid representation learning	UniProtKB database	2021
	mCNN-ETC [62]	Position-specific scoring matrix (PSSM)	UniProtKB database	2022
	DeepEfflux [65]	Position-specific scoring matrix (PSSM)	Transporter Classification Database	2018

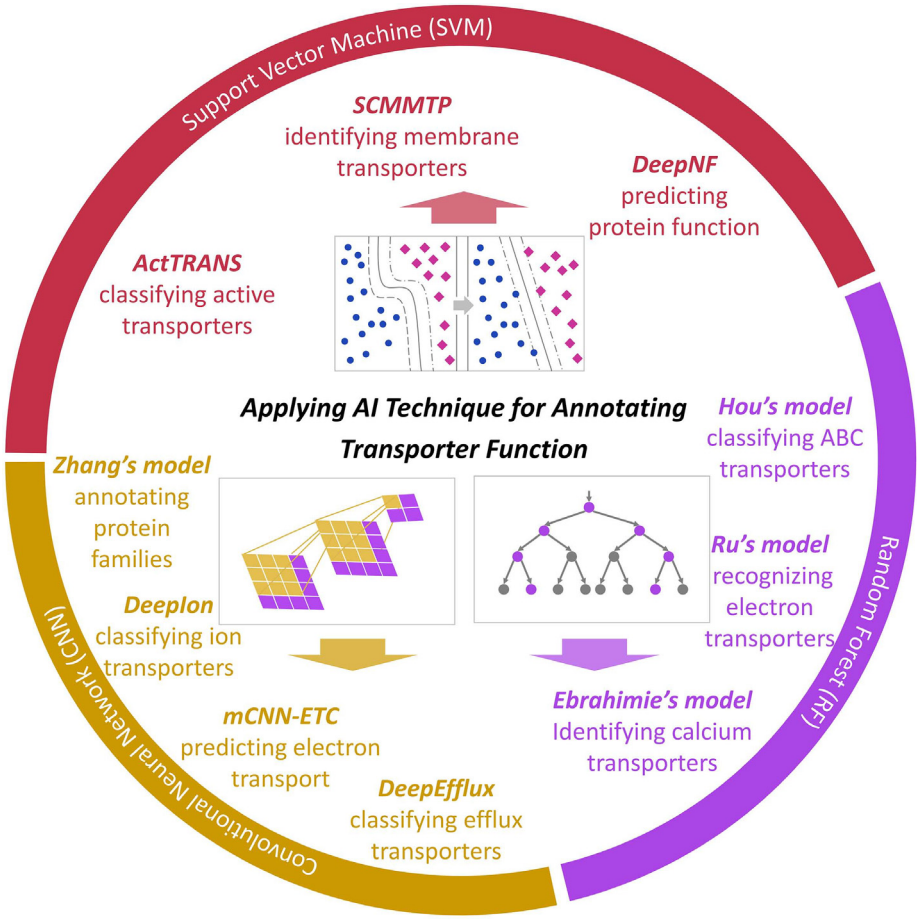

Fig. (2). The principle of the widely used artificial intelligence approaches in the field of transporter functional annotation and their specific application models. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

DeepNF [50] is a method utilizing the SVM algorithm to classify functional labels of proteins and is a multimodal deep autoencoder-based method for extracting high-level characteristics of proteins from multiple networks of heterogeneous interactions. Owing to the deep learning technology behind the method, DeepNF allows for a more accurate capture of associated protein signatures from a complicated network of non-linear interactions.

2.2. Random Forest (RF)

The RF is a supervised ML algorithm trained on a dataset of the same size as the training set, built from random resampling of the training set itself [51]. It is robust to overfitting, which has been popularly used in the classification of imaging data [52, 53], ADME prediction [54], and disease classification [55, 56], and is

more stable in high-dimensional parameter spaces than other machine learning. Recent models using the RF algorithm for transporter classification and function prediction are shown below.

Hou *et al*. [57] applied RF as a significant classifier to classify ATP-binding cassette (ABC) transporter proteins and employed 188D as a feature extraction method based on sequence and physicochemical attributes. Finally, the average accuracy of the training and test sets was 89.54% and 89%, respectively. The findings showed that a combination of the 188D feature extraction method and the RF algorithm would effectively identify the ABC transporter.

Ebrahimie *et al*. [40] compared the classification and numerical characteristics of general and specific calcium transporters with a random forest algorithm and an attribute-weighted model. Based on 5-fold cross-validation, the model achieved an accuracy of 88.88% along with an area under the curve (AUC) of 0.964. In addition, the study demonstrated that decision trees using accuracy criteria could predict the specific calcium transporters independent of organismal and sub-cellular location.

Ru *et al*. [58] used a combination of RF and distance-based Top-n-gram feature extraction methods to recognize electron transporters. The prediction accuracy of electron transport protein reached 82% and 86% in cross-validation and independent testing, respectively. This tool is considered to be an effective one for the identification of electron transporters.

2.3. Convolutional Neural Network (CNN)

CNN is a feed-forward neural network with deep structure and convolutional computation. It is one of the representative algorithms of deep learning designed for processing structured data arrays, such as image-related tasks [59-61]. In particular, CNN has been focused on the functional annotation of transports, which extract features of amino acids by convolutional operations [62]. The following are the recent models using the CNN algorithm for transporter classification and function prediction.

DeepIon [63] was designed to automatically classify ion transporters and ion channels from membrane proteins *via* training a deep neural network with hidden features extracted from a position-specific scoring matrix (PSSM) as input. In addition, DeepIon allows for the use of unbalanced datasets, and the best features are selected for the normalization method based on the proposed model, which can effectively improve classification performance. During cross-validation, the predicted ACC values for ion channels, ion transporters, and other proteins were 87.05%, 87.49%, and 94.35%, respectively, and the MCC values for the predicted three were 0.75, 0.75, and 0.89, respectively.

Zhang *et al*. [64] developed a CNN model based on amino acid characterization learning, using a limited number of feature proteins to explore the properties of annotated protein families by considering amino acid position information. The average precision and recall reached 0.976 and 0.977, respectively, demonstrating that this method outperformed most existing rival methods without using any human-designed features.

mCNN-ETC (62) is designed to predict electron transport proteins and classify the corresponding complexes. This deep learning model works by transforming the evolutionary information of a protein into image-like data consisting of twenty channels corresponding to the twenty amino acids involved in the protein sequence. The study constructed CNN hidden layers with varying scan windows in parallel to strengthen the detection capability of the model. The performance achieved an accuracy of 97.41%.

DeepEfflux [65] is a DL model that combines 2D convolutional neural networks and PSSM as an input in the classification of efflux transporters. The accuracy of the independent test is 96.02%, showing that DeepEfflux outperforms other traditional competing models in identifying efflux transporter families.

These AI techniques not only show excellent performance in the construction of tools for predicting transporter function but are also well applied for guidance in experiments. Yoshinobu *et al*. [66] predicted the function of glucose transporters (GLUTs) by deep learning methods, culminating in experimental validation of biomolecules that regulate GLUT1 function, revealing potential molecular targets for the treatment of myocardial ischemia. Felicia *et al*. [67] applied machine learning tools to investigate the stability and structural changes of SLC6A1 encoded by γ-aminobutyric acid (GABA) transporter 1 (GAT-1) and verified that the SLC6A1 variant could cause myoclonic atonic epilepsy (MAE). Machine learning-based functional prediction of membrane carnitine transporter OCTN2 variants to aid in the diagnosis and interpretation of variants in the treatment of Carnitine Transporter Deficiency (CTD) was made by Megan *et al*. [68]. The application of AI techniques for predicting functional annotation of transporters has greatly facilitated the research of disease mechanisms and the discovery of drug targets.

3. DEVELOPING AI MODELS FOR RECONSTRUCTING TRANSPORTER STRUCTURE

The membrane proteins, which comprise approximately 30% of the human proteome, enable communication between cells and between cells and extracellular environments [69, 70]. Due to their important functions in biosignal communication, membrane proteins have always been the major research topic in the field of drug target discovery [71-73]. And the membrane proteins are thus the primary therapeutic target of about 60% of approved drugs [74-76]. In recent years, many complex membrane protein structures have been resolved due to cryo-Electron Microscopy (cryo-EM) [77], including the ABC transporter (breast cancer resistance protein, P-glycoprotein, *etc.*) [78], proton-coupled folate transporter (PCFT; SLC46A1) [79], amino acid transporter LAT1 (SLC7A5) [80], and so on. Cryo-EM has become a routine method for resolving structures ranging from large biological assemblies to small biomolecules with resolutions approaching true atomic levels. The protein structure data resolved from cryo-EM makes the application of AI techniques for transporter structure prediction possible [81]. However, the experimental characterization of some membrane protein structures is exceptionally difficult, owing to their hydrophobic surfaces as well as their lack of conformational stability [75, 82-84], and the resolution of most cryoelectron microscopy transporter structures is insufficient to describe molecular interactions useful for rational drug design, rendering many transmembrane proteins poorly described. Thus, there is an urgent need to use AI methods to distinguish and characterize transmembrane proteins with currently available data [85]. The following section illustrates the application of AI techniques in transporter structure prediction, and the detailed description of the AI algorithms, code availability, and the datasets adopted for the specific method is displayed in Table **2**.

AlphaFold [27] is a deep-learning algorithm that can predict protein structures with atomic-level accuracy even in the absence of similar structures. Its predictive performance shows accuracy comparable to experimental structures in most cases and greatly exceeds that of other methods. Due to the complexity of membrane protein structures, AlphaFold2 was proposed to predict the complex structure of the transporter. Diego *et al*. introduced AlphaFold2 for the conformational study of the Amino Acid-Polyamine-Organocation (APC) transporter structure [86]. Janaszkiewicz *et al*. utilized the structure of the inward conformation of hOAT1 predicted by AlphaFold2 to investigate the effects of polymorphisms and mutations on hOAT1 and understand the substrate binding mode [87].

RoseTTAFold [88] is applied for structure predictions of protein using a three-track neural network, which outperforms Al-

Table 2. Artificial intelligence tools and models applied to transporter structure discovery. A detailed description of the AI algorithms, the datasets adopted for the specific method, and code availability are described.

Model	AI Method	Database(s) Employed	Code Availability	Year
AlphaFold [27]	Novel neural network (Evoformer)	PDB Database	https://github.com/deepmind/alphafold	2021
P2Rank [32]	Random forest	PDB Database	http://github.com/rdk/p2rank	2018
TorchMD [89]	Deep neural network	QM9 data set	http://github.com/torchmd	2020
TopSuite [90]	Deep neural network	PDB Database	https://cpclab.uni-duesseldorf.de/topsuite/	2021
DESTINI [91]	Convolutional residual neural network	PISCES library	http://pwp.gatech.edu/cssb/destini	2018

phaFold with high accuracy. Compared with AlphaFold2, it requires fewer computational resources, making it more widely used in transporter structure and function prediction. The network can also rapidly generate accurate models of protein-protein complexes based on sequence information, thereby reducing the time required by traditional methods.

P2Rank [32] was designed to rapidly and accurately predict the ligand binding site from the protein structure. The tool is out-of-the-box, independent of other bioinformatics tools or databases, and can directly process the structure of multiple chains to discover potential ligand binding sites consisting of residues from multiple strands. The speed and fully automated predictive capabilities of the model make it especially suitable for processing large datasets or as part of a scalable structural bioinformatics pipeline.

TorchMD [89] is a deep-learning computational framework designed to perform molecular simulations with a combination of classical and ML algorithm capabilities. The framework allows for end-to-end training and learns and simulates the coarse-grained models of protein folding, which is considered an effective tool that can be used for molecular simulation.

The TopSuite web server [90] was proposed, which consists of two parts: protein model quality assessment (TopScore) and template-based protein structure prediction (TopModel). The first part provides a meta-prediction of global and residue-based model quality assessment *via* deep neural networks, while the second part uses a top-down consensus approach to predict protein structures to aid template selection, followed by refinement and evaluation of the predicted structures using TopScore.

DESTINI [91] is a new computational method that integrates deep learning algorithms with template-based structure modeling for the prediction of protein residue/residue contacts. The model successfully predicts a larger number of previously intractable protein tertiary structures, demonstrating significantly improved predictive power for the first time in a massive tertiary structure prediction of more than 1200 single-domain proteins.

Different from the protein structure prediction tool above, SidechainNet [92] is an ML-based all-atom protein structure dataset that not only includes angular and atomic coordinate information that can describe each protein structure for all heavy atoms but can also be extended by the user to include newly published protein structures. Sidechain Net is proving to be a helpful dataset for exploring the representativeness of studied proteins and is very helpful for predicting protein structure-property relationships or predicting protein-protein as well as protein-ligand interactions.

Transporter structures predicted by AI technology are used to support experimental research. Ole *et al*. [93] applied machine learning and experimental transporter data to predict OCT1 structure and confirm substrate activity by *in vitro* uptake assays. It contributes to performing more targeted screening in drug development. Zhai *et al*. [94] analyzed the structure of SotB and SotB2 predicted by AlphaFold2 and explained the different molecular mechanisms of substrate recognition between SotB and SotB2. The structural information in this study and a biochemical examination provide a valuable framework for further deciphering the functional mechanisms of SotB and its family. Ritika *et al*. [95] predicted the three-dimensional structure of P-glycoprotein by deep learning, analyzed its binding stability to drugs, and then verified the effect of the protein on parasite drug resistance through animal experiments.

4. PREDICTING DRUG-TRANSPORTER INTERACTIONS USING AI TECHNOLOGY

The transporters dictate the absorption, distribution, metabolism, and excretion (ADME) of both endogenous substances and specific drugs [1] and have great implications on drug pharmacokinetics and toxicokinetics [17]. A number of important drug transporters, such as P-glycoprotein, breast cancer resistance protein, multidrug and toxin extrusion proteins, organic anion transporter 1/3, organic cation transporter 2, organic anion transporting polypeptide 1B1/1B3, play a crucial function in the disposition and toxicity of many approved drugs [20]. And these important transporters are usually of great clinical interest for their ability to modulate potential drug-drug interactions [10]. In other words, when multiple drugs are used in combination, drugs may compete with each other for binding to the same transport protein, which could result in an unanticipated variation in drug concentration and possible adverse drug reactions [1, 96].

Therefore, the identification of the relationship between transporters and drugs plays a vital role in circumventing undesirable drug-drug interactions (DDIs) as well as facilitating novel drug development [28]. With the accumulation of high-quality data on various drugs and transporters in current knowledgebases, such as VARIDT [97, 98], Metrabase [99], and the improvements in related AI technologies, a large number of AI tools have been developed to predict multiple transporter-drug specificities in studies. In this section, a summary of the advantages and accuracy of these tools was conducted. A detailed description of the AI algorithms, the feature extraction methods, and the datasets adopted for the specific methods are described in Table **3**. As well as the AI methods and their principles for the prediction of transporter-substrate and transporter-inhibitor are illustrated in Fig. (**3**).

4.1. Applying AI Methods for Transporter Substrates Prediction

Several transporter-drug specificity prediction models consider only one type of transporter at a time, such as Li *et al*. [100] proposed a Naive Bayes (NB) classifier for predicting potential P-glycoprotein substrates, and Hazai *et al*. [101] constructed an SVM classification model for predicting breast cancer resistance protein substrates. The others took advantage of available data on drug transporters to predict the relationship of drugs to multiple transporters, as shown in the following section.

Table 3. The prediction of transporter-drug interactions with artificial intelligence technology. A detailed description of the AI algorithms, the feature extraction methods, and the datasets adopted for the specific methods are provided.

Type	Model	AI Method	Feature Extraction Methods	Database(s) Employed	Year
Transporter-substrates prediction	STS-NLSP [102]	Random Forest	Structural fingerprints and biological information	Metrabase, ChEBI	2019
	TranCEP [103]	Support Vector Machine	Amino acid composition, pairwise amino acid composition, and pseudo-amino acid composition	UniProtKB database	2020
	Nguyen's model [104]	Support Vector Machine	Word embedding approach and frequencies of protein biological words	UniProtKB database	2019
	Li's model [105]	Support Vector Machine	Integrating features from position-specific score matrix, PROFEAT, and Gene Ontology	UniProtKB database	2016
	TrSSP [106]	Support Vector Machine	Amino acid composition, dipeptide composition, physicochemical composition, biochemical composition, and PSSM	UniProtKB database	2014
	DEEPScreen [107]	Convolutional Neural Network	2D representations generated by RDkit	ChEMBL database	2020
	DeepConv-DTI [108]	Convolutional Neural Network	Performing convolution on amino acids subsequences to capture local residue patterns of generalized protein classes	DrugBank, KEGG, IUPHAR	2019
	DeepACTION [109]	Convolutional Neural Network	Dipeptide composition, amino acid composition, CTD, pseudo amino acid composition, autocorrelation, quasi-sequence-order	DrugBank, KEGG	2020
Transporter-inhibitors prediction	Kong's model [110]	RF, SVM, logistic regression, k nearest neighbor (KNN)	Extracting characteristics of the molecules by CDK, Graph, MACCS, and PubChem	ChEMBL database	2020
	Kharangarh's model [111]	KNN, SVM, RF	Features/descriptors were generated by PyDPI	Metrabase database	2018
	Lee's model [112]	RF, eXtreme gradient boosting	Descriptors generated by RDkit, and pharmacophore descriptors calculated by Gobbi	ChEMBL database	2021
	Khuri's model [113]	KNN, SVM, PLS, RF, Recursive Neural Networks	2D representations generated by RDkit	Literature review	2018

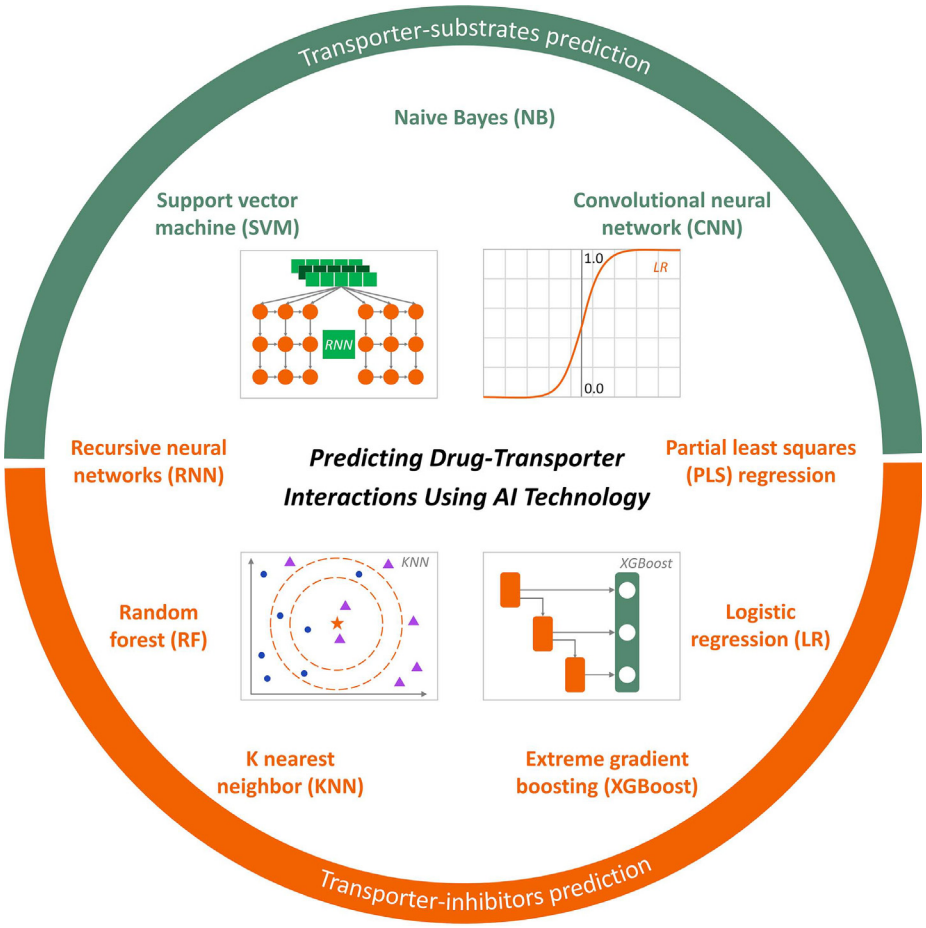

Fig. (3). Artificial intelligence methods and their principles for the prediction of drug-transporter interactions. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

STS-NLSP [102] was designed to predict whether a substrate is specifically recognized by one or more of the following transporters (ABCG2; MDR1; MRP1; MRP2; MRP3; MRP4; NTCP2; SLC15A1; SLC22A1; SLCO1A2; SLCO1B1; SLCO1B3; SLCO2B1) with the use of the substrate's structural fingerprint and chemical ontology information. A hybrid model based on similarity features was constructed using the network label space partitioning method and combined two-dimensional fingerprinting and semantic similarity to perform effectively for transporter-substrate specificity prediction.

TranCEP [103] was designed as a tool for predicting the substrates of membrane transport protein. Support vector machines are used as classifiers in this tool and integrate information on amino acid composition, location, and evolution. The model can achieve an overall accuracy and MCC of 69.23% and 0.69%, respectively. Moreover, Nguyen *et al*. [104] proposed an SVM classifier combined with word embedding techniques to identify the substrate specificity of transporters. In this method, the word embedding and frequency of biological words are defined as sequence features of the protein. The mean AUC of the tool reached 0.96 and 0.99 in the 5-fold cross-validation and test sets, respectively. Li *et al*. [105] developed an SVM-based model to predict the substrate specificity of transporter proteins by combining PSSM, PROFEAT, and Gene Ontology (GO) features. The overall accuracy of the method was 96.16% and 80.45% for the cross-validation of the baseline and independent datasets. TrSSP [106] is a computational model based on SVM with protein sequence characteristics (including PSSM, amino acid, dipeptide, physicochemical, and biochemical composition) for predicting the substrate specificity of 7 transporter categories: anions, amino acids, cations, electrons, proteins/RNAs, glycans and other transporters. The average overall prediction accuracy reached 78.88%, and the MC reached 0.41 on the independent dataset.

In addition, there are partial studies that used AI technology to predict drug-target interactions (DTI), for example, DEEPScreen [107], which applied deep convolutional neural networks and off-the-shelf 2D structural characterization of compounds to produce highly accurate DTI predictions; DeepConv-DTI [108], which adopted a deep learning-based approach that captures local residues in protein; and DeepACTION [109], which is proposed to employ deep learning along with the correct representation of its protein feature descriptors for predicting potential DTIs. These deep learning-based tools can predict a large number of new DTIs and provide comprehensive information to motivate scientists to develop novel drugs.

4.2. Utilizing AI Methods for Transporter Inhibitors Prediction

Kong *et al*. [110] developed 16 prediction models to predict SERT inhibitors using four ML tools, including SVM, RF, logistic regression (LR) and K-nearest neighbors (KNN), and four molecular fingerprints, consisting of CDK, Graph, MACCS, and PubChem. LR and KNN are both machine learning algorithms, with LR typically used to solve binary classification problems, while KNN determines the category to which the majority of the K most similar samples in the feature space belong, as shown in Fig. (**3**). This study identified 12 molecular structures that are frequently found in SERT inhibitors, providing an essential guide for the design of SERT inhibitors.

Moreover, Kharangarh *et al*. [111] applied multiple ML algorithms (*e.g*., SVM, RF, and KNN) to a dataset of 124 inhibitors and 115 non-inhibitors for the prediction of multidrug resistance-associated protein-2 transporter (MRP2) inhibitors. The final results were prediction accuracies of 76%, 72%, and 79% on the training set, cross-validation set, and external set, respectively. In addition, Lee *et al*. [112] developed a reverse screening platform targeting the dopamine transporter (DAT) and human ether-a-go-go (hERG) binding. The approach integrated an ML (eXtreme gradient boosting and RF)-based quantitative structure-activity relationship (QSAR) model and successfully identified a pair of structural elements of DAT inhibitors with opposite binding affinity trends for DAT and hERG. EXtreme gradient boosting (XGBoost) is an integrated algorithm for machine learning, combining gradient boosting and decision tree-related elements, and the method is able to achieve stronger learning by integrating multiple weak learners.

Khuri *et al*. [113] developed a series of ML models consisting of Recursive Neural Networks (RNN), KNN, SVM, RF, and multivariate partial least squares (PLS) regression, to predict inhibitors of transporter proteins in the liver. The RNN is one of the deep learning algorithms designed to recognize patterns in data that carry information from the past. In other words, an RNN learns from the past and processes new data based on experience and is more versatile and powerful than feedforward neural networks, as described in Fig. (**3**). The results showed that the area under the receiver operating curve for these models varied between 67% and 78% and performed similarly in internal cross-validation datasets, while the RF and SVM models performed best in external validation.

AI techniques were applied to guide the design of substrate or inhibitor experiments for the transporter. Sabrin *et al*. [114] screened for human glucose transporter 1 (hGLUT1) inhibitors using AI technology, followed by biosynthesis to determine the biological activity of the compounds, which may be a potential target for cancer therapy. Inhibitors of the uptake transporter OATP1B1 (SLC01B1) were predicted using the SVM approach and tested *in vitro* by Thomas *et al*. [115]. It showed that 15 of the 19 compounds predicted to be active were found to have inhibitory activity. Oriol *et al*. [116] used deep learning and *in vivo* validation of sodium-glucose co-transporter 2 inhibitor expression to improve cardiomyocyte death for discovering new treatments for heart failure.

5. SUMMARY AND PROSPECT

A comprehensive literature review and a summary were conducted in this study from three aspects of AI application, including transporter classification and functional annotation, structure discovery, and drug transporter relationship prediction. Firstly, the principles and characteristics of the main AI methods currently applied to these three areas were briefly described. Secondly, the specific application scenarios, functions, and accuracy of the various models were described in detail. Thirdly, the advantages of these AI methods applied in the field of the transporter were summarized. Although AI has been applied in the field of the transporter in great numbers and with promising results, at this stage, AI has not disrupted the traditional system of functional and structural studies of transporters, and there are still some limitations.

5.1. Limitations of AI in Transporter-centered Functional and Pharmacological Research

Firstly, there is the limitation of high-quality data. AI algorithms cannot be developed without being driven by data. Accurate and high-quality data can sometimes make a simple model better than a complex one. In terms of transporter research, there are many excellent publicly accessible databases. For example, the TCDB [35] is the only officially recognized database for the transporters classification and contains information on the structure, function, and biotechnology of transporters from different species, the VARIDT [97, 98], which provides information on all human drug transporters and all aspects of their variability, and the Metrabase [99] which includes information on substrates and modulators associated with the human transporters. However, the data volume is

insufficient to allow for more sophisticated and highly accurate studies. The acquisition of high-quality data is a vital issue for complicated biological systems. Differences in batches of data, collection methods, and collection locations can make it difficult to convert data into consistent and valid data. Secondly, the predictions of models trained together with data from *in vitro* experiments are often unconvincing due to variations in the actual situation. In addition, the imbalance in the extant data often leads to decreased model accuracy. With advancing experimental techniques and improved data processing methods, higher quality data is expected to be available to improve the true applicability of the model and facilitate the clinical translation of the data.

The second limitation is the interpretability of the model. Deep learning is commonly thought of as a "black box", *i.e*., the outcome is visible but not the cause of the result [117]. In the case of functional annotation of transporters, although DL methods can predict the function of a particular protein, the computational process involved is unknown, and the basis for classification is uncertain, making the majority of predictions unacceptable when accuracy is unreliable. Therefore, in subsequent transporter-centered functional and pharmacological studies, the interpretability of the models should be enhanced as much as possible while ensuring their accuracy to better find the basis for classification or relevant mechanisms.

5.2. Implications of Transporters in Cell Communication and Bioengineering

The main focus of this review is on the pharmacological significance of transporters. However, as a specific class of membrane proteins, the functional annotation and classification of transporters and structural discoveries also play an important role in intercellular/ inter-organ communication and bioengineering [118-120]. In particular, in the field of intercellular/interorgan communication, there is growing evidence that transporters can mediate remote communication between different cells/organs by regulating endogenous metabolites and signaling molecules and maintaining homeostasis in the organism (*i.e*., remote sensing and signaling hypothesis). A typical illustration, in the context of renal disease, is that the function of the intestinal uric acid transporter ABCG2 becomes significantly essential [121, 122]. When declining renal function interferes with the normal renal elimination of this potentially toxic organic anion, remote communication occurs between the dysfunctional kidney and the intact gut to regulate uric acid levels, thereby avoiding more severe renal and non-renal pathology due to high uric acid levels [120]. Therefore, a comprehensive grasp of transporter-mediated endogenous metabolites and signaling molecules is fundamental to the understanding of cellular, organ, inter-organ, and inter-organismal communication and the mechanisms that maintain homeostasis in the organism [1, 123, 124].

In addition, in the field of bioengineering, transporters have gained widespread attention due to their substrate specificity and potential to significantly improve the performance of microbial cell factories [118]. There have been successful cases where the Gal2 transporter was coupled to xylose isomerase so that when xylose was absorbed, it was guided to xylose isomerase, reducing by-product formation and increasing ethanol production [125]. In contrast, the leaked intermediate products were reintroduced into the cells from the medium by *E. coli* uptake transporters PotE and GabP, respectively, to increase the production of the pathway product glutaric acid [126]. Therefore, an understanding of the substrate uptake or efflux by the transporter and a comprehensive knowledge of its function is crucial for the microbial cell factory and is of great importance for improving productivity and increasing economic efficiency [119, 127]. Although these studies are beyond the scope of this review, an understanding of these areas could enhance the overall insight into the transporter and benefit the application of transporters in the field of pharmacology.

CONCLUSION

All in all, this review provides a detailed summary of current AI algorithms and tools in the field of transport and describes the main features of these traditional machine learning and deep learning algorithms, as illustrated in Table **4**. These research efforts are expected to guide the discovery of novel transporters, novel drug development, and the avoidance of clinical drug-drug interactions.

Table 4. Traditional machine learning algorithms and deep learning algorithms are applied to the field of the transporter, and the key features of these AI algorithms are shown below.

Type	AI Method	Applications in the Field of Transporter	Key Features
Traditional machine learning algorithms	Support Vector Machine (SVM)	Transporter functional annotation; prediction of drug-transporter interactions	Wide application, a simple algorithm suitable for small sample learning in the field of transporter
	Random Forest (RF)	Transporter functional annotation; prediction of drug-transporter interactions; transporter structure discovery	High performance, capable of picking out important features and working with unbalanced data
	Naive Bayes (NB)	Prediction of drug-transporter interactions	Simple algorithm, suitable for small sample data, with more stable classification efficiency
	Logistic Regression (LR)	Prediction of drug-transporter interactions	Frequently used for classification tasks, short training time, and good model interpretability
	K Nearest Neighbor (KNN)	Prediction of drug-transporter interactions	Easy to implement, short training time, suitable for multi-category classification
	Partial Least Squares (PLS) Regression	Prediction of drug-transporter interactions	Suitable for problems with multiple features and collinearity, available for dimensionality reduction
	eXtreme Gradient Boosting (XGBoost)	Prediction of drug-transporter interactions	Support parallelism, by learning multiple classifiers to get the best classifier, higher model accuracy
Deep learning algorithms	Convolutional Neural Network (CNN)	Transporter functional annotation; transporter structure discovery; prediction of drug-transporter interactions	Automatic feature extraction, suitable for high-dimensional data, widely used in image recognition
	Deep Neural network (DNN)	Transporter structure discovery	High performance, more computational power, and higher computational cost
	Residual Neural Network (RNN)	Prediction of drug-transporter interactions	Suitable for solving sequential problems with temporally dynamic behavior

AUTHORS' CONTRIBUTIONS

Zeng and Zhu participated in the research design, Yin, You, Lu, and Li performed data analysis, and Zeng, Zhu, Yin, You, and Li contributed to the writing of the manuscript.

LIST OF ABBREVIATIONS

ABC	=	ATP-binding Cassette
ADME	=	Absorption, Distribution, Metabolism, and Excretion
AI	=	Artificial Intelligence
AUC	=	Area Under the Curve
CNN	=	Convolutional Neural Network
DDI	=	Drug-drug Interaction
DL	=	Deep Learning
DTI	=	Drug-target Interactions
GO	=	Gene Ontology
Metrabase	=	The Metabolism and Transport Database
ML	=	Machine Learning
NLP	=	Natural Language Processing
NLSP	=	Network Label Space Partitioning
OAT1	=	Organic Anion Transporters 1
PSSM	=	Position-specific Scoring Matrix
RF	=	Random Forest
SVM	=	Support Vector Machine
VARIDT	=	Variability of Drug Transporter Database

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

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